Shonle

On the Nutritive Value of Glutamic and Aspartic Acids for the Maintenance of Adult Mice

ON THE NUTRITIVE VALUE OF GLUTAMIC AND ASPARTIC ACIDS FOR THE MAINTENANCE OF ADULT MICE

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Horace Abbott Shonle

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TABLE OF CONTENTS

ON THE NUTRITIVE VALUE OF GLUTAMIC AND ASPARTIC ACIDS FOR THE MAINTENANCE OF ADULT MICE.

I. INTRODUCTION.

The failure of a single natural food or of a mixture of foods to support growth or maintenance may be due to a quantitative or qualitative deficiency of proteins, to a low or unfavorable mineral content, to a lack of accessory dietary substances, or to the presence of toxic substances.[1] In the series of experiments to be reported, the limiting factor was the qualitative deficiency of the protein intake.

No doubt is now entertained that proteins are broken down in the alimentary tract into their constituent amino-acids which are absorbed as such. Recent experiments reported by Abderhalden[2] have shown that dogs could be kept in nitrogen balance for as long a period of time as 147 to 290 days when the sole source of nitrogen was hydrolyzed protein. Therefore we would expect that if each amino-acid or group of related amino-acids possessed a specific function, their complete or practically complete removal must lead to serious nutritive disturbances.

The experimental work reported below was undertaken in order to determine the question of the indispensability to the mammalian organism of glutamic and aspartic acids for maintenance.

Three general methods of determining the indispensability of amino-acids have been used. One, first employed by Abderhalden and Henriques and perfected by Abderhalden, is based on the fact that animals can maintain a nitrogen equilibrium or even grow when the nitrogen requirements are covered by the digestion products secured from proteins hydrolyzed by enzymes or by enzymes and acids until a positive biuret test is no longer obtained or until

the peptide linkages are entirely destroyed. In the former case, presumably some of the simpler polypeptides were present in addition to the amino-acids. Abderhalden removed different amino-acids as completely as possible from such mixtures and determined the nutritive value of the remaining mixtures by feeding them to animals in rations containing no other nitrogenous substances though complete in every other respect. If the residual amino-acids were inadequate, the final proof of the indispensability of the withdrawn amino-acids was made by the addition of the removed amino-acids in a pure state. If the addition did not give favorable results, no conclusions could be reached, in as much as there might have been the unintentional removal of other substances, or the residual amino-acids themselves might have undergone a change incidental to the removal, either of which circumstances might have exerted a harmful effect on the nutritive value of the residual mixture.

The second general method has been extensively used by Osborne and Mendel with encouraging results. These investigators have fed carefully purified proteins incorporated into an otherwise adequate, synthetic, basal ration whose nitrogen and protein content is minimal. In this way the differences in the nutritive value of many proteins have been shown to vary directly with the qualitative or quantitative differences of the amino-acid content. The unsatisfactory knowledge of the amino-acid content of the proteins, upon which the success of this method depends, has limited its scope. Also, proteins deficient in one or more amino-acids are scarce, and there may be further complications due to some peculiar proportion of those amino-acids which do occur in them, or to marked differences in their digestibility.

The third method used by Abderhalden[3] and Mitchell[4] consists of feeding definite mixtures of isolated amino-acids incorporated in rations of minimal nitrogen content. Though this is the most direct and logical way of

attacking the problem, the expense and difficulty of preparing the acids must be considered. Up to the present, no definite mixture of isolated amino-acids has been discovered which has been satisfactorily shown to cover the nitrogen requirements of growing or adult animals for indefinite periods of time. Until such a mixture has been found, this method is of no practical significance for a problem of the character of that with which this paper is concerned.

In those cases, where the amino-acids under investigation can be practically quantitatively removed from the hydrolyzate, the former Abderhalden method recommends itself. Since such a removal of the di-basic amino-acids is possible by the methods described below, this method of procedure was followed.

Hopkins and Foreman[5] have contributed some experimental data indicating that glutamic and aspartic acids are not essential for growth. Though they give the weight curves of two rats which, on a synthetic ration free from glutamic and aspartic acids, grew for a period of 35 days, no mention is made whether these data are representative of a series of experiments or comprise the sole experimental evidence. The amino-acid mixture used was prepared from casein, hydrolyzed by boiling for 40 hours with 25 percent sulfuric acid and freed from the glutamic and aspartic acids by the method of Foreman.[6] This mixture constituted the sole source of nitrogen in an otherwise adequate synthetic ration.

Abderhalden was unable to secure any clear cut results with a mixture of amino-acids from which the glutamic acid had been removed as the hydrochloride, due to the difficulty of removing the residual hydrochloric acid without destroying the tryptophane.

II. EXPERIMENTAL INVESTIGATION.

THE SELECTION OF EXPERIMENTAL ANIMALS.- Though the use of the larger experimental animals, such as dogs, permits the determination of the nitrogen balance, yet unless such determinations can be carried out over long continued periods of time, qualitative deficiencies in the ration fed may not be indicated.[3] The unpalatability of the ration, the ensuing digestive disturbances, and the difficulty experienced in the preparation of the food materials renders long continued experiments with dogs very difficult. That qualitative or quantitative deficiencies, however, can be shown only after a long period of time has been brought out by the work of Osborne and Mendel[7] and Wheeler.[8] These investigators found that the rations which appeared adequate for short periods may ultimately lead to nutritive failure and that the necessity of long continued observations in nutrition may be dependent upon the slow depletion of the body store of the essential factors. Hence animals must be selected which will consume the required rations for continued periods of time.

Mice were selected for this experiment partly because they are omnivorous and hardy, and partly because they require such a small amount of food, a factor which permits the use of larger numbers of animals. Unless the qualitative or quantitative food requirements of mice (or rats) are indicative, in a general way, of the food requirements of all mammalian organisms, experiments with such animals are of no general value. The extensive use in the past decade, of rats and mice, in experiments to determine the nutritive value of a wide variety of substances, by Osborne and Mendel, Abderhalden, McCollum and associates, Mitchell, Hogan, Hopkins and associates, and many others, renders further discussion of this point unnecessary, particularly since close similarities in growth, metabolism, and food requirements have been shown to exist be-

tween these animals and other mammals.[9]

It has been stated that the span of life for the white mouse is two years and that they are sexually mature in six to eight weeks, though according to Robertson[10] they continue to grow slowly for sixty weeks. Since the stages of growth and maturity are completed in such a short time, experimental observations on the effects of a particular dietary may be made over a relatively long period of the life of the animal. Because of the short life span, marked changes in the nutritive equilibrium will manifest themselves more quickly than in the larger animals.

DETAILS OF THE EXPERIMENT.- The mice were kept in round galvanized iron cages, about eight inches in diameter and six inches in height, either separately, in pairs, or occasionally in threes. Clean paper excelsior was always kept in the cages.[11] Distilled water was supplied through the removable top of the cage, by an inverted bottle provided with a bent delivery tube extending a few inches into the cage. By using a tube of 6 mm. bore which was bent at an angle of 120 degrees and had one end somewhat constricted, no trouble was experienced in keeping the tube full of water. The mice were kept in light dry quarters at a temperature of 70 to 80 degrees from October to May. The cages were cleaned out frequently and sterilized every few weeks by passing through hot alkali.

The food was placed in small porcelain crucibles which were fastened to the side of the cage by means of a wire loop. In order to catch the scattered food, tin plates 4 to 5 inches in diameter were placed under the crucibles. As a further precaution, the cages were placed over glass crys-

[11] The paper excelsior used, analyzed 0.80 percent ash and 0.05-0.06 percent nitrogen.

tallizing dishes or granite plates which contained sheets of filter paper to facilitate the good separation of food waste and urine. In this way it was possible to secure a good determination of the food intake, which, as Osborne and Mendel point out, is very essential. The food was weighed out once a day at which time the amount of uneaten food in the crucible was determined. Fresh food was placed in the cages every day except Sunday.

The mice were weighed, at first, every other day; but when it became evident that the experiments must be of several months duration, weekly weighings were considered sufficient, unless a change of ration or condition of the animal made it desirable to follow the weight changes more closely.

PREPARATION OF FOOD MATERIALS.- The method of preparing the amino-acids was simple. Commercial casein, or the mixed protein, secured from skim milk powder by precipitation in aqueous solution with tri-chloro-acetic acid, was completely hydrolyzed by boiling with ten times the weight of 25 percent sulfuric acid continuously for 30-35 hours.[12] The sulfuric acid was then

[12] The completion of hydrolysis was taken as that point at which the alpha-amino nitrogen, determined by the Van Slyke method, practically reached a constant value.

quantitatively precipitated by adding a sufficient amount of a boiling saturated solution of barium hydroxide with the avoidance of any excess. The barium sulfate was filtered off and thoroughly washed with boiling water and both the filtrate and the wash water were evaporated to dryness at a low temperature and the residue powdered. In order to secure comparable results, the glutamic and aspartic acids were removed from a portion of each preparation. The method of Foreman[6] was followed.

The Foreman method is based on the fact that the calcium salts of the di-basic organic acids have a much higher degree of insolubility in water

and alcohol than the calcium salts of the mono-basic acids. The protein is completely hydrolyzed with hydrochloric acid and the latter removed as completely as possible in vacuo. The solution of amino-acid hydrochlorides is then made alkaline with calcium hydroxide, filtered, and reduced to a definite volume. Then, on the addition of 97 percent alcohol, the di-basic amino-acid salts rapidly precipitate out and are filtered off and freed from calcium with oxalic acid. This fraction contains the glutamic and aspartic acids as such, some glutamic acid in the form of pyrrolidonecarboxylic acid, and small amounts of substances of unknown composition which can be precipitated from neutral solution by the addition of silver sulfate and phosphotungstic acid.

In as much as there was no mineral acid present, in the residual mixtures of mono-basic amino-acids used in this work, the calcium was removed as the carbonate instead of as the oxalate, by repeatedly saturating the hot aqueous solution with carbon dioxide. The filtrate from the calcium carbonate, containing the mono-basic amino-acids, was evaporated to dryness and the residue powdered. In this way it was possible to remove as high as 30 percent of the total weight of the original amino-acid mixture, an amount which compares with the results of Hopkins and Foreman.

Tryptophane was prepared from casein, in part by the method of Homer[13] and in part by the method of Hopkins and Cole.[14] The latter method gave the better results. The tryptophane was fed as such on crystallizing from alcohol or as the calcium salts of carbamino acids as prepared by the method of Siegfried and Neumann.[15] The latter method of separation recommends itself, in that it is easier to separate the tryptophane from the impurities of the mother liquor than is possible by direct crystallization.

Cystine was prepared from wool according to the method of Folin as

modified by Lewis.[16]

The "protein-free" milk was first prepared according to the directions of Osborne and Mendel and contained 0.64 to 0.74 percent of nitrogen, part of which is doubtless protein nitrogen which could not be removed by the method of preparation. In order to reduce this nitrogen, the "protein-free" milk used in the latter period of experimentation was prepared by the trichloro-acetic acid method of Mitchell and Nelson[17] with the omission, however, of the second filtration after boiling. This preparation contained 0.44 percent of nitrogen.

The butter fat was prepared from the best creamery butter according to the directions of Osborne and Mendel.[18]

The sugars, starch, and lard used were commercial products of good quality.

BASAL RATIONS FOR MAINTENANCE.- Since growth, in all probability, sets a higher standard in regard to the amino-acid requirement than does maintenance, the best point of attack in determining nutritive requirements would appear to be the effect of the amino-acids upon maintenance rather than on growth. The basal ration of Osborne and Mendel, consisting of protein 18 percent, "protein-free" milk 28 percent, starch 26 percent, lard 10 percent, and purified butter fat 18 percent has been shown by Mitchell, Wheeler and others to be capable of maintaining mice for long periods of time. Mitchell was unable to secure maintenance of mice with the McCollum and Davis ration of dextrin, lactose, purified butter fat, agar-agar, protein and a synthetic salt mixture. Neither did the ration used by Hopkins and associates consisting of starch, sugar, lard, purified butter fat, hydrolyzed casein, ash of dog biscuit and oats, and accessory dietary substances maintain the mice successfully. The latter point was confirmed in this experiment. Therefore, in

all of the experiments reported in this paper modifications of the Osborne and Mendel rations were employed.

FEEDING EXPERIMENTS WITH MIXTURES OF AMINO ACIDS.- The mice used in these experiments varied in age from two to eight months. Part were albinos and part were colored mice secured from the Genetics Division through the courtesy of Dr. J. A. Detlefsen. The change in weight of the animals, the survival period, and the general well being were taken as indications of the effect of the ration upon the animal. Though changes in body weight may be due to gain or loss of water or fat, such a criticism is not valid in prolonged experiments.[19]

In all the experiments on the indispensability of the amino-acids under investigation, control mice were fed rations containing the complete amino-acid mixtures, and their weight and survival period was used as a standard with which to compare the mice on the incomplete ration. If an incomplete ration appeared inadequate, the animal was placed on the complete ration or on one in which the incomplete mixture was made complete by the addition of the removed amino-acids in a pure condition. Consideration must be given to the difficulty which exists in bringing animals, whose vitality is lowered through prolonged periods of experimentation, from a low plane of nutrition to a higher one.

In view of the trouble experienced by Mitchell in inducing mice to consume the Osborne and Mendel ration, in which an 18 percent mixture of isolated amino-acids was substituted for the protein, it was considered essential to find if better results could not be secured with a different percent of amino-acids. Three concentrations were used, a 12 percent, a 15 percent, and the 12 percent ration " diluted" with 33 percent of a non-nitrogenous ration resembling the Osborne and Mendel ration. In this latter case, in order to assure

a sufficient nitrogen intake, a 2.5 percent mixture of amino-acids was added to the water. The 15 percent ration was found to give the best results. An attempt was made to use the tasteless calcium salts of carbamino acids prepared from the complete amino-acid mixture in the ration for the nitrogen requirement. This effort was unsuccessful since the mice refused to eat a sufficient amount of the ration.

Ration A.

		Percent
Amino-acids,		12.0
(a) from casein or milk proteins,	11.5 pct.	
(b) tryptophane,	0.25 "	
(c) cystine	0.25 "	
Sucrose or lactose,		6.0
Starch,		26.0
"Protein-free" milk,		28.0
Purified butter fat,		18.0
Lard,		10.0
		100.0

Ration B.

		Percent
Amino-acids,		15.0
(a) from casein or milk proteins,	14.5 pct.	
(b) tryptophane,	0.25 "	
(c) cystine,	0.25 "	
Sucrose or lactose		12.0
Starch,		17.0
"Protein-free" milk,		28.0
Purified butter fat,		18.0
Lard,		10.0
		100.0

Ration C was identical with Ration B except that the "protein-free" milk was prepared by the tri-chloracetic acid method. Those rations in which the amino-acid mixture was complete, are designated in the description of experiments below by the number 100, while those which lack the di-basic amino-acids are designated by the number 120.

In order to enable the mice to adapt themselves more easily to the experimental diet, they were placed for several days on a preliminary ration similar to Rations A and B except for the fact that they contained casein instead of the amino-acids.

Since maintenance is dependent in part on the energy intake, unless this intake is sufficient, changes which may occur in the body weight cannot be attributed to the qualitative or quantitative differences of the nitrogen intake in a ration otherwise adequate. In the work reported by Mitchell, it

was shown that the daily food consumption by adult mice of rations containing 28 percent fat must be at least 8 grams per 100 grams of body weight to insure satisfactory maintenance. In connection with the experiments here reported, it was found that when the food intake of a mouse on an adequate ration was 8 to 10 grams per 100 grams of body weight, maintenance was secured. With these data it is possible to distinguish the declines in weight due to an insufficient energy intake from those due to deficiencies of the nitrogen intake.

The Feeding of Complete Mixtures of Amino-acids.

Another point to be demonstrated was the ability of the mice to maintain themselves when their sole source of nitrogen was supplied by a mixture of amino-acids prepared by the acid hydrolysis of casein. Until recently, it was not considered possible to maintain life with the supplemented products secured from the complete hydrolysis of proteins.

The most successful of the experiments demonstrating the adequacy of a complete mixture of amino-acids for maintenance were run on three male and two female mice.

The three male mice, 100a ♂, 100b ♂, and 100c ♂ weighed 23.4, 25.1, and 24.2 grams, respectively, when they were removed from the preliminary ration and placed on Ration 100A which contained 12 percent of a complete mixture of amino-acids. During the first 15 days that they were on this ration, their daily food consumption averaged 6.6 grams per 100 grams of body weight, and their body weight declined. With the hope of increasing their food consumption, the percentage of amino-acids was lowered from 12 to 9 by the addition of a non-nitrogenous ration in which starch replaced the amino-acids. In order to insure an adequate nitrogen consumption, a 2.5 percent mixture of amino-acids was placed in the water. This procedure, which was adopted on the 16th day, did not increase the food intake and the loss in weight continued.

On the 48th day, Mouse 100a ♂ had lost 34 percent of its weight, Mouse 100b ♂ 40 percent, and Mouse 100c ♂ 34 percent. The physical condition of the mice was poor, they showed a marked weakness, often trembled violently, and their fur was rough.

At the end of the 54th day, the addition of the non-nitrogenous ration was discontinued and the original ration 100A was replaced by ration 100B which contained 15 percent of amino-acids. A few days later the addition of the amino-acids to the water was discontinued.

On this ration the average daily food intake increased to 10 grams per 100 grams of body weight and the mice seemed to have completely recovered from their former poor condition. At the end of the 82nd day, Mouse 100b ♂ showed a gain of 30 percent of its weight 34 days previous with a complete return to its normal appearance. After holding its weight for 29 days longer it died at a weight of 17.5 grams, having been on these rations for 111 days. Up to the time of its death, the animal appeared to be in good nturitive condition.

Mouse 100c ♂ showed a similar improvement of appearance on changing to ration 100B, and at the end of the 70th day had gained 15.6 percent of its weight 22 days previous. After maintaining this weight for 16 days, the animal was placed on a ration lacking the di-basic acids.

Mouse 100a ♂ likewise improved in appearance on changing to ration 100B, but was unable to increase its weight. It maintained itself at 15 to 16 grams from the 48th to the 156th day of the experiment before showing the decline which preceded its death on the 180th day. During the last 40 days of the experiment the "protein-free"milk was prepared by the trichloroacetic acid method. Table I gives the weights of the mice together with the average daily food intake per 100 grams of body weight.

TABLE I.

Days.	Weight in grams of Mouse. 100a ♂	100b ♂	100c ♂	Average daily food intake in grams per 100 grams of body weight.	
0	23.4	25.1	24.2	0.0	Ration 100A,
8	22.5	21.1	23.1	6.5	(12 pct. amino-acids.)
14	20.0	19.6	20.4	6.6	
20	18.2	17.4	18.6	7.7	Ration 100A,
32	18.0	16.6	18.4	6.0	"diluted".
48	15.5	15.0	16.0	6.8	
54	15.0	14.7	16.2	7.7	
62	14.5	16.7	17.3	7.9	Ration 100B,
70	14.8	17.6	18.5	8.9	(15 pct. amino-acids.)
82	15.5	19.5	18.5'	9.8	
100	16.0	19.7		10.0	
111	15.5	17.5 died		11.5	
128	15.3			10.0	
142	15.0			7.4	Ration 100C,
156	15.0			12.5	("protein-free" milk prepared
170	13.7			12.1	by trichloroacetic acid).
180	11.5 died			10.0	

'Mouse 100c ♂ was removed on the 86th day at a weight of 17.8 grams.

The second experiment was undertaken with two female mice, 110a ♀ and 110b ♀, in order to determine the possibility of feeding the tasteless calcium salts of the carbamino-acids as a source of nitrogen. A ration, in which these salts were included in amounts equivalent to 12 percent of the amino-acids, was fed for 8 days. Due to an almost absolute refusal to eat the food, at the end of this period the mice had lost 28 and 30 percent of their original weights of 20.3 and 23.1 grams, respectively. Thereupon they were placed on ration 100A, and at the end of the 16th day they showed an increase in weight due to the consumption of more food. When 33 percent of the non-nitrogenous ration was incorporated in ration 100A and 2.5 percent of amino-acids added to the water, both the body weight and the food intake fluctuated, with the result that at the end of the 54th day of the experiment, the

mice were in practically the same low nutritive state as when first removed from the ration containing the calcium salts of the carbamino-acids.

When this procedure was discontinued and the mice were placed on ration 100B, containing 15 percent of amino-acids, a rapid increase in weight and food intake occurred accompanied by a return to normal appearance. On the 74th day, Mouse 110a ♀ weighed 19.0 grams - almost its original weight - while Mouse 110b ♀ weighed 19.3 grams. At the end of the 86th day Mouse 110b ♀ was removed from this ration and placed on a ration containing only mono-basic amino-acids. Mouse 110a ♀, at the end of the 117th day was placed on a ration similar to 100A except that the "protein-free" milk was replaced by starch and the ash of dog biscuit and oats. It was unable to maintain itself on this ration and was subsequently placed on other rations until its death on the 207th day of experimental feeding, during which time the amino-acid mixtures comprised the sole source of nitrogen. The weights and average food consumption are given in Table II.

TABLE II.

Days.	Weight in grams of Mouse. 110a ♀	110b ♀	Average daily food intake in grams per 100 grams of body weight.	
0	20.3	23.1	0.0	Ration containing calcium salts of the carbamino-acids.
8	14.5	16.2	1.6	
14	15.6	18.3	8.9	Ration 100A, (12 percent amino-acids.)
20	16.2	16.0	6.1	Ration 100A, "diluted".
32	15.8	16.7	5.7	
40	16.7	17.2	7.1	
54	15.3	15.0	5.5	
62	17.2	15.8	7.4	Ration 100B, (15 percent amino-acids.)
74	19.0	19.3	10.2	
86	16.5	18.3 removed	7.7	
100	19.7		11.2	
117	16.8 removed		7.9	

In a third series of experiments, three partially grown male mice

were placed on the ration containing 15 percent of the complete amino-acids mixture (Ration 100B). The initial weights of Mice 140a ♂, 140b ♂, and 140c ♂ were 16.6, 17.8, and 11.8 grams respectively. During the first three weeks that they were on this ration, they suffered a decline in weight of 23 percent, 31 percent, and 21 percent, respectively, even though their daily food intake was close to 10 grams per 100 grams of body weight. From the 21st to the 101st day of experimental feeding, the three mice maintained their weights at this level with a rather constant food intake sufficient to meet all energy requirements. The substitution of the "protein-free" milk prepared by the use of the trichloroacetic acid for that prepared by the method of Osborne and Mendel on the 78th day was not accompanied by any marked change in weight or food intake. On the 102nd day, the mice were changed to a ration containing the mono-basic amino-acids only. Table III gives the data for this experiment.

TABLE III.

Days.	Weight in grams of Mouse. 140a ♂	140b ♂	140c ♂	Average daily food intake in grams per 100 grams of body weight.	
0	16.6	17.3	11.8	0.0	
12	13.7	14.7	10.4	9.5	
20	12.7	12.2	9.3	7.9	Ration 100B.
38	12.1	12.3	10.2	10.9	(15 percent amino-acids.)
52	11.7	11.8	10.2	10.5	
66	12.2	12.2	10.7	10.7	
73	12.5	12.5	10.7	9.5	
80	12.2	12.0	10.0	9.9	
94	13.2	13.2	10.6	9.7	
101	12.0	12.3	10.0	10.1	

removed all mice

In order to test this point further, Mouse 101 ♂ of an initial weight of 20.5 grams was placed on ration 100B. On the 16th day it showed a decline of 24 percent although the daily food intake had been high. This level in weight was held until the 40th day, at which time the animal was

placed on a ration containing starch and the ash of oats and dog biscuit instead of "protein-free" milk. A steady decline in weight followed until the animal died on the 106th day of feeding at a weight of 10.5 grams. During the latter portion of the period, the animal was weak and appeared emaciated. The data secured are shown in Table IV.

TABLE IV.

Days.	Weight in grams of Mouse 101 ♂.	Average daily food intake in grams per 100 grams of body weight.	
0	20.5	0.0	Ration 100B, (15 percent amino-acids.)
12	16.5	9.7	
16	15.5	10.0	
24	14.7	9.1	
33	14.8	9.4	
44	15.2 removed	10.2	

The last two experiments carried out on two male mice, 105a ♂ and 105b ♂ were less successful than the others. The first mouse died at the end of 23 days after declining from 25.5 to 17.0 grams, and the second died at the end of 41 days after a decline from 18.5 to 14.0 grams. The physical condition of the animals was good up to the time of their death.

The most important point to be noted in these experiments is the ability of the animal organism to maintain itself, or even increase in weight, when the nitrogenous requirements are covered by amino-acid mixtures, secured by completely hydrolyzing casein or milk proteins with mineral acids, and supplementing the resulting mixtures of amino acids with cystine and tryptophane. Mice 100a ♂, 140a ♂, 140b ♂, and 140c ♂ maintained their weight for 81 to 101 days, while Mice 100b ♂, 100c ♂, 110a ♀, and 110b ♀ show the ability of the animal organism to grow as well as to maintain itself under these conditions. Until the recent work of Hopkins and Abderhalden, it was not thought possible that animals could maintain their weight or grow when their

source of nitrogen was the amino-acid mixture secured from the acid hydrolysis of proteins. The fact that maintenance of life is possible under such conditions indicates that the only indispensable amino-acids destroyed in the acid hydrolysis of proteins are cystine and tryptophane.

The Osborne and Mendel ration containing 15 percent of amino-acids gave the best results, and no difference in maintenance could be detected when the ration contained the "protein-free" milk prepared by the use of trichloroacetic acid.

The Feeding of Mixtures of Mono-basic Amino-acids.

The second group of experiments was designed to test the nutritive value of glutamic and aspartic acids. Osborne and Guest[20] found 15.5 percent of glutamic and 1.4 percent of aspartic acid in casein. Foreman,[6] using an entirely different method, found 24 percent of di-basic amino-acids in casein. By following the latter procedure as outlined above it was found possible to remove crude glutamic and aspartic acids equal to 28-30 percent of the hydrolyzate from casein or the combined milk proteins.

As mentioned above, there is the possibility that some essential substance might be removed with the calcium salts of the di-basic acids by the Foreman method and might be contained in the unidentified precipitate secured by the addition of silver sulfate and phosphotungstic acid. It was hoped that this criticism might be overcome by securing the same results on the addition of pure glutamic and aspartic acids to the residual mono-basic mixture of the amino-acids as were secured by the use of the complete mixture, but due to lack of time this was not thoroughly tested out. There is no possibility of the entrance of toxic substances or of a change in the character of the remaining amino-acids due to the chemical manipulations, in which only pure calcium oxide,

redistilled alcohol, and carbon dioxide were used. Hence any difference which may occur on changing from the complete amino-acid mixture to the mono-basic or vice-versa must be ascribed, - almost entirely, at least, - to the di-basic amino-acid content.

Since some criticism attaches itself to the work of Hopkins and Foreman because of the small number of animals and the short duration of the periods of observation, the following experiments were planned to correct these points. The results secured on the rations containing the mono-basic mixtures of amino-acids were compared with those secured from the first group. The rations employed were in every way identical with those employed in the first group of experiments with the exception of the amino-acid content, which was the same in percentage, but lacked the glutamic and aspartic acids and any other material which may have been removed with them. In some cases, the mice were kept on the rations containing the mono-basic amino-acids until they succumbed in order to compare their survival period with that of the first group; in others alternations were made with the rations containing the mixture of complete amino-acids.

In only one case did the longevity of an animal on the mono-basic amino-acid ration approach that of the mice on the complete amino acid ration. Three female mice, 122a ♀ , 122b ♀ , and 122c ♀ , with an initial weight of 30.0, 26.5 and 31.5 grams, respectively, were placed on a ration containing a 12 percent mixture of the mono-basic amino-acids (Ration 120A) "diluted" with 33 percent of the non-nitrogenous ration. A 2.5 percent mixture of the amino-acids was added to the water. The mice, which had been in a high nutritive condition, rapidly declined in weight. Mouse 122b ♀ died at the end of the 14th day after losing 53 percent of its weight, while Mouse 122a ♀ died on the 30th day after a decline of 43 percent of its weight. Mouse 122c ♀ showed a more gradual decline of 36 percent in 34 days. At the end of the 22nd day, the

original ration was replaced by a ration containing 15 percent of mono-basic amino-acids (Ration 120B), and at the same time the addition of the amino-acids to the water was discontinued. The food intake of Mouse 122c ♀ began to increase, until at the end of the 34th day it averaged 10 grams per 100 grams of body weight per day, - a level which was maintained until a few days before its death.

At the end of the 55th day, the mouse had gained 5.3 grams over its weight 21 days previous. During the next seven days a decline in weight commenced which continued until its death.[21] During the decline in weight,

[21] It may be noted that shortly after the decline set in, the Osborne and Mendel "protein-free" milk was replaced by that prepared with trichloroacetic acid.

the animal dropped from 25.5 to 15.0 grams, although the food intake was constant. There were several periods during which maintenance was kept for a time, the most noticeable being for a period of 35 days at a level of 19.0 grams. During the last few days of the experiment, 4.0 percent of glutamic and 0.5 percent of aspartic acids were added to the ration, but even this did not check the decline and the animal died at a weight of 15.0 grams after having been on experimental rations for 146 days. The animal appeared emaciated and trembled violently towards the end of the experiment. The results are given in Table V.

TABLE V.

Days.	Weight in grams of mouse. 122a ♀	122b ♀	122c ♀	Average daily food intake in grams per 100 grams of body weight.	
0	30.0	26.5	31.5	0.0	Ration 120A "diluted". (12 percent of mono-basic amino-acids).
10	20.8	15.0	26.2	3.4	
14	20.0	12.5 died	24.7	4.7	
22	19.0		21.7	5.6	
30	17.2 died		21.7	6.4	Ration 120B (15 percent of mono-basic amino-acids).
34			20.2	13.6	
46			23.2	11.6	
55			25.5	11.2	
66			23.0	8.5	
88			19.5	11.2	
95			20.2	10.1	
109			19.3	10.0	
123			19.0	10.8	
130			17.3	12.0	
137			16.5	8.4	
146			15.0 dead	9.1	

A similar experiment was carried out with two more female mice, 120a ♀ and 120b ♀, with initial weights of 24.5 and 29.0 grams, respectively. They were placed on the same ration as were the mice in the last experiment for the first 45 days, after which time Ration 120B was given them. Mouse 120b ♀ slowly dropped to 54 percent of its weight in 35 days and then maintained itself at this level until its death on the 42nd day of experimentation. At this time it weighed 13.5 grams, showing a loss of 53 percent in weight. Mouse 120a ♀ slowly dropped to 65 percent of its initial weight in 35 days and maintained itself at this level until its death on the 69th day. The average daily food intake was in the neighborhood of 7.5 grams per 100 grams of body weight. Both mice showed evidences of malnutrition through weakness and trembling. Table VI gives the results of this series.

TABLE VI.

Days.	Weight in grams of Mouse. 120a ♀	120b ♀	Average daily food intake in grams per 100 grams of body weight.	
0	24.5	29.0	0.0	Ration 120A "diluted".
7	20.0	22.5	7.8	
19	17.3	19.2	6.5	
35	15.8	15.7	6.0	
42	16.0	13.5 dead	6.4	
53	15.0		7.6	Ration 120B.
68	15.2 dead		10.3	

Two mice, 125a ♂ and 125b ♂, with initial weights of 26.4 and 23.5 grams, respectively, were placed on the 15 percent amino-acid ration. They showed the usual decline accompanied by a low food intake at the start. Mouse 125a ♂ declined in weight steadily until it died, after being on the ration 72 days, at a weight of 11.2 grams. During the latter portion of the period the condition of the animal was very poor, - its coat was rough, it trembled and was weak.

At the end of the 30th day Mouse 125b ♂ had lost 36 percent of its weight and then maintained itself until the 72nd day. At the end of this time the mouse, which was in a very poor physical condition, was placed on Ration 100C containing 15 percent of the complete mixture of amino-acids. The animal's appearance was bettered, and it increased in weight from 14.5 to 17.5 grams in 7 days, but fell back to 14.3 grams on the 95th day. Upon being placed on the mono-basic mixture again, the mouse declined in weight to 12.8 grams. An attempt was made to check this decline by adding 4 percent of glutamic and 0.5 percent of aspartic acids to the ration in place of an equal amount of the mono-basic mixture. This attempt was unsuccessful due, possibly to the fact that the animal did not have the necessary vitality.[22] The animal died, after having

[22] In several cases it was found impossible to bring an animal back to normal weight or even keep it alive on natural food, if it had been kept on experimental rations too long.

been on an experimental diet for 119 days, at a weight of 10.2 grams. Table VII gives the data secured for the above experiment.

TABLE VII.

Days.	Weight in grams of Mouse. 125a ♂	125b ♂	Average daily food intake in grams per 100 grams of body weight.	
0	26.4	23.5	[illegible]	Ration 120B. (15 percent of mono-basic amino-acids).
11	18.9	18.5	4.4	
22	18.0	16.2	7.6	
30	16.7	15.0	11.5	
58	12.5	15.0	10.4	
72	11.2 dead	14.5	10.5	
79		17.5	11.4	Ration 100C. (15 percent of complete amino-acid mixtures).
95		14.3	9.0	
103		12.8	10.1	Ration 120C.
110		12.2	8.7	Ration 120B plus glutamic and aspartic acids.
119		10.2 dead	7.4	

Although Mouse 125b ♂ was able to maintain its weight for 40 days, it was in a poor physical condition, and upon changing it to the ration containing the di-basic amino-acids, an improvement of condition was secured and a temporary increase in weight resulted. The daily food intake remained almost constant.

Mouse 110b ♀ , it will be remembered, was removed from the ration containing 15 percent of the complete mixture of amino-acids and placed on one containing the same amount of the mono-basic acids. For a period of 12 days, on the former ration, a weight of 18 to 19 grams had been maintained. Though the food intake on the mono-basic acid ration was more than sufficient to meet the energy requirement, the animal lost 3.1 grams in 70 days. Towards the last of this period maintenance was secured and when the former complete amino-acid ration was resumed no increase in weight occurred. When, at the end of the 94th day, the animal was placed back upon the mono-basic acid ration,

a decline in weight set in which could not be checked by the feeding of the complete ration and the animal died on the 112th day after having been on experimental rations in all for a period of 198 days. The results are given in Table VIII.

TABLE VIII.

Days.	Weight in grams of Mouse. 110b ♀	Average daily food intake in grams per 100 grams of body weight.	
0	18.3	0.0	Ration 120B. (15 percent mono-basic amino-acids.)
9	16.3	9.4	
20	18.3	11.7	
42	15.7	10.8	
70	15.2	11.6	
84	16.0	13.0	Ration 100B. (15 percent complete mixture amino-acids.)
90	15.0	12.2	
98	13.2	11.9	Ration 120B.
101	13.0	6.9	
105	13.1	6.9	Ration 100C.
112	10.3 dead	6.2	

Another experiment was carried out on Mouse 124a ♂, whose initial weight was 27.5 grams. When placed on ration 120B, the animal showed the usual sharp decline in weight and poor food intake. Although at the end of 15 days the average daily food intake had increased to 10 grams per 100 grams of body weight, this decline continued, consisting of a gradual dropping from one level to another, until the mouse died at the end of 82 days at a weight of 14 grams. The physical condition of the animal during this period was good, and scarcely any signs of malnutrition were evident even at the time of its death, although it had lost 49 percent of its original weight. The data are included in Table IX.

TABLE IX.

Days.	Weight in grams of mouse. 124a ♂	Average daily food intake in grams per 100 grams of body weight.	
5	27.5	6.9	Ration 120B. (15 percent mono-basic amino-acids)
11	19.2	5.1	
22	20.0	10.8	
30	17.8	12.7	
44	17.2	10.9	
58	15.2	11.9	
65	13.5	11.9	
79	13.7	12.7	
82	14.0 dead	7.9	

In contrast to the above results, are the rapid declines in weight, leading to an early death, which occurred in the following seven cases. The ration fed in every case contained the 15 percent mixture of mono-basic amino-acids. These results were secured at different times and with several consecutive preparations of amino-acids.

The food intake in all these cases was rather low, as was the usual result during the first few weeks, but both the relative and the absolute daily food intake showed a tendency to increase with the increasing time the animals were on the ration. Usually the animals were in a poor physical condition at the time of their death. Table X gives the condensed results of this series of experiments.

TABLE X.

Mouse.	Beginning of Experiment.		End of Experiment.		
	Weight in grams.	Aver. food intake per 100 grams body weight at end of first week.	Time. days.	Weight. grams.	Aver. food intake per 100 grams of body weight.
123 ♂	23.0	9.4 g.	22	14.0	8.6 g.
124 ♀	23.3	5.0	13	16.0	11.6
127a ♂	25.8	6.5	43	13.7	7.3
127b ♂	16.0	6.5	33	9.0	6.8
128a ♂	20.8	5.7	43	9.0	9.6
128b ♂	18.3	5.7	33	8.8	7.1
128c ♂	27.0	5.7	61	11.8	11.8

In the case of Mouse 128c ♂, an unsuccessful attempt was made to check the decline by the addition of glutaric and aspartic acids to the ration on the 43rd day.

Three male mice, 130a ♂, 130b ♂, and 130c ♂, with an initial weight of 22.7, 21.3 and 17.3 grams, respectively, were placed on the twelve percent mono-basic acid ration. Very rapid declines occurred, and at the end of 13 days, the mice had lost 24, 30 and 18 percent, respectively, of their weights. Mouse 130b ♂ died at this point. Upon changing to the "diluted" 12 percent ration containing the complete amino-acid mixture, the rapid declines in the weight of the remaining two mice were checked. When on the 45th day a change was made to the 15 percent amino-acid ration, a slight increase of weight was secured.

Mouse 130a ♂ died on the 70th day, having lost 29 percent of its original weight, and Mouse 130c ♂ died at the end of 69 days having lost 19 percent of its original weight. The significant point to be noted is the slowing up of the decline in weight at a point where it normally does not slow up on the mono-basic ration, as will be seen by reference to the summary tables given below. The data are given in Table XI.

TABLE XI.

Days.	Weight in grams of Mouse. 130a ♂	130b ♂	130c ♂	Average daily food intake in grams per 100 grams of body weight.	
0	22.7	21.3	17.3	0.0	Ration 120B.
7	19.0	17.2	14.8	4.5	
13	17.2	15.0 dead	14.2	5.5	Ration 100A, "diluted".
17	18.0		13.8	6.9	
25	17.1		14.1	6.6	
45	15.5		12.2	7.5	
65	17.7		13.6	8.2	Ration 100B.
69			13.6	13.0	
70	16.2 dead		dead		

Mouse 123a ♂ with an initial weight of 18.5 grams, was placed on the 15 percent mono-basic amino-acid ration. The animal consistently declined in weight, losing 45 percent of its original weight during the 53 days it was on the ration, even though the average daily food intake was 10 grams per 100 grams of body weight. Upon changing to the 15 percent complete amino-acid ration, an increase in weight of 2.6 grams was secured by the 81st day. When a change was made at this time to the mono-basic ration, a decline set in so that at the end of 22 days, the mouse only weighed 10.0 grams. An unsuccessful attempt was made to bring the animal back to a higher weight by the addition of glutamic and aspartic acids. The mouse finally died at the end of the 122nd day at a weight of 8.3 grams. Table XII gives the data for this experiment.

TABLE XII.

Days.	Weight in grams of mouse. 123a ♂	Average daily food intake in grams per 100 grams of body weight.	
0	18.5	[illegible]	Ration 120B.
11	14.0	8.9	
22	13.2	9.4	
37	11.5	11.3	
51	10.2	11.5	
65	12.0	9.2	Ration 100C.
81	12.8	11.3	
86	11.7	7.2	Ration 120C.
100	10.0	8.9	
110	9.5	9.5	Ration 120C plus glutamic and aspartic acids.
122	8.3 dead	9.0	

Another example of the inadequacy of the mono-basic amino-acids for maintenance is shown by the inability of the three mice, 140a ♂, 140b ♂, and 140c ♂ to hold their weight when changed from the ration containing the complete amino-acid mixture (see Table III, p. 15) to the one with the mono-basic amino-acids. They were removed from the former ration on the 101st day. Table XIII gives the results secured.

TABLE XIII.

Days.	Weight in grams of Mouse. 140a ♂	140b ♂	140c ♂	Average daily food intake in grams per 100 grams of body weight.	
0	12.6	12.3	13.0	0.0	Ration 120F.
7	10.5	10.7	11.0	8.8	
14	10.5	11.0 dead	9.0	7.8	
21	9.4		8.3	8.1	
34	8.5		7.7	6.2	

The most striking results on this phase of the experiment were secured with Mouse 100c ♂, which was removed, at a weight of 17.8 grams, from the ration containing the complete amino-acid mixture (see Table I, p. 13) and placed upon the mono-basic acid ration. Though, after this change, the average daily food intake was as high as 16.9 grams per 100 grams of body weight, the weight of the animal steadily declined to 14.0 grams on the 42nd day. Upon being placed on the former ration again, the animal increased its weight from 14.0 grams to 18.8 grams in 10 days, although the food intake was somewhat diminished. At this point, i.e., the 52nd day, the animal was removed and placed on another ration composed of a different mixture of amino acids. It is of interest to note that the increase still continued. The results are given in Table XIV.

TABLE XIV.

Days.	Weight in grams of the Mouse. 100c ♂	Average daily food intake in grams per 100 grams of body weight.	
0	17.8	0.0	Ration 120B.
9	17.0	11.9	
28	15.3	16.5	
35	14.8	16.1	
42	14.0	16.9	
49	18.5	16.0	Ration 100B.
52	18.8 removed[1]	15.4	

[1] A weight of 21.0 grams was reached on the other ration in the course of 4 days.

III. SUMMARY AND CONCLUSION.

The maintenance secured with animals on the ration containing the complete amino-acid mixture served as the normal from which the effects of the rations deficient in the di-basic amino-acids were measured. Then, since it is evident from the data represented above that the differences in the maintenance secured with the complete and the mono-basic amino-acid rations are relative, a comparison of the average results secured from each of the above groups of experiments will be of value. Table XIV gives the average weights of the mice for 5 or 10 day periods in percent of their initial weights on both the complete and the mono-basic amino-acid rations.

TABLE XIV.

WEIGHTS OF MICE IN PERCENT OF INITIAL WEIGHT.

Mice on Complete Amino-acid Ration.

Mouse.	Initial weight in grams.	Days. 0	5	10	15	20	25	30	40	50	75	Survival period in days.
100a ♂	23.4	100	97	88	84	78	80	80	75	65	64	180
100b ♂	25.1	100	88	82	76	69	72	69	66	60	72	111
100c ♂	24.2	100	94	89	84	77	79	80	74	67	77	208"
110a ♀	20.3	100	(76	76)'	78	80	70	79	82	70	92	207"
110b ♀	23.1	100	(78	77)'	77	69	69	70	75	64	83	189"
140a ♂	16.6	100	91	83	78	77	72	75	73	71	75	131"
140b ♂	17.8	100	95	86	79	69	68	70	69	67	70	115"
140c ♂	11.8	100	88	89	81	79	76	79	87	85	89	140"
101 ♂	20.5	100	87	80	77	71	72	74	74	(removed)		116"
105a ♂	25.5	100	--	81	74	74	--	67				32
105b ♂	18.5	100	--	98	85	84	--	79	76			42
Average	20.6 g.		91	86	79	75	74	75	75	69	77	ext.133 days

TABLE XIV. (CONTINUED)

WEIGHTS OF MICE IN PERCENT OF INITIAL WEIGHT.

Mice on Mono-basic Amino-acid Ration.

Mouse.	Initial weight in grams.	Days 0	5	10	15	20	25	30	40	50	75	Survival period in days.
122a ♀	30.0	100	78	69	67	65	57	57				30
122b ♀	26.5	100	79	57	47							18
122c ♀	31.5	100	88	83	73	72	66	69	70	75	71	145
120a ♀	24.5	100	87	78	74	70	69	67	57	63		70
120b ♀	29.0	100	82	67	67	67	50	57	49			42
125a ♂	26.4	100	80	72	70	68	--	63	59	51		72
125b ♂	23.5	100	86	79	76	69	--	64	68	62	62	119'''
124a ♂	27.5	100	76	70	71	73	--	65	62	56	50	82
123 ♂	23.0	100	80	70	63	61						22
124 ♀	23.3	100	83	73								13
127a ♂	25.8	100	82	72	--	64	60	56	52			43
127b ♂	16.0	100	84	75	--	63	58	56				35
128a ♂	20.8	100	77	63	--	55	54	--	50			43
128b ♂	18.3	100	85	67	--	59	55	46				31
128c ♂	27.0	100	87	69	--	69	66	--	57	48		61
130a ♂	22.7	100	85	79	(removed)							70'''
130b ♂	21.3	100	86	82								13
130c ♂	17.3	100	87	80	(removed)							67'''
123a ♂	18.5	100	80	76	77	71	--	61	62	55	(removed)	122'''
Average	23.3 g.		83	72	69	66	60	60	59	62	**61 pct.**	**48 days**

' Period when mice were on calcium carbamino salts; not used in calculations.

"Survival period of mice initially fed a ration containing the complete amino-acid mixture for periods of 44 to 117 days, and then transferred to other rations containing an incomplete mixture of amino acids or salts.

"'Survival period of mice transferred from ration containing the mono-basic amino-acid mixture to that containing the complete mixture. Not included in general average.

The low average initial weight of the mice on the complete amino-acid ration is due to the fact that the mice in Experiment 140 were only partially grown. When the weights of these mice are excluded an average weight of 23.8 grams is secured.

Since, as pointed out above, the food intake is a factor to be considered, the average daily food intake in grams per 100 grams of body weight for 5 or 10 day periods for each of the groups is given in Table XV. Chart I gives a graphic presentation of the above results.

TABLE IV.

THE AVERAGE FOOD INTAKE IN GRAMS PER 100 GRAMS OF BODY WEIGHT.

Mice on Complete Amino-acid Mixtures.

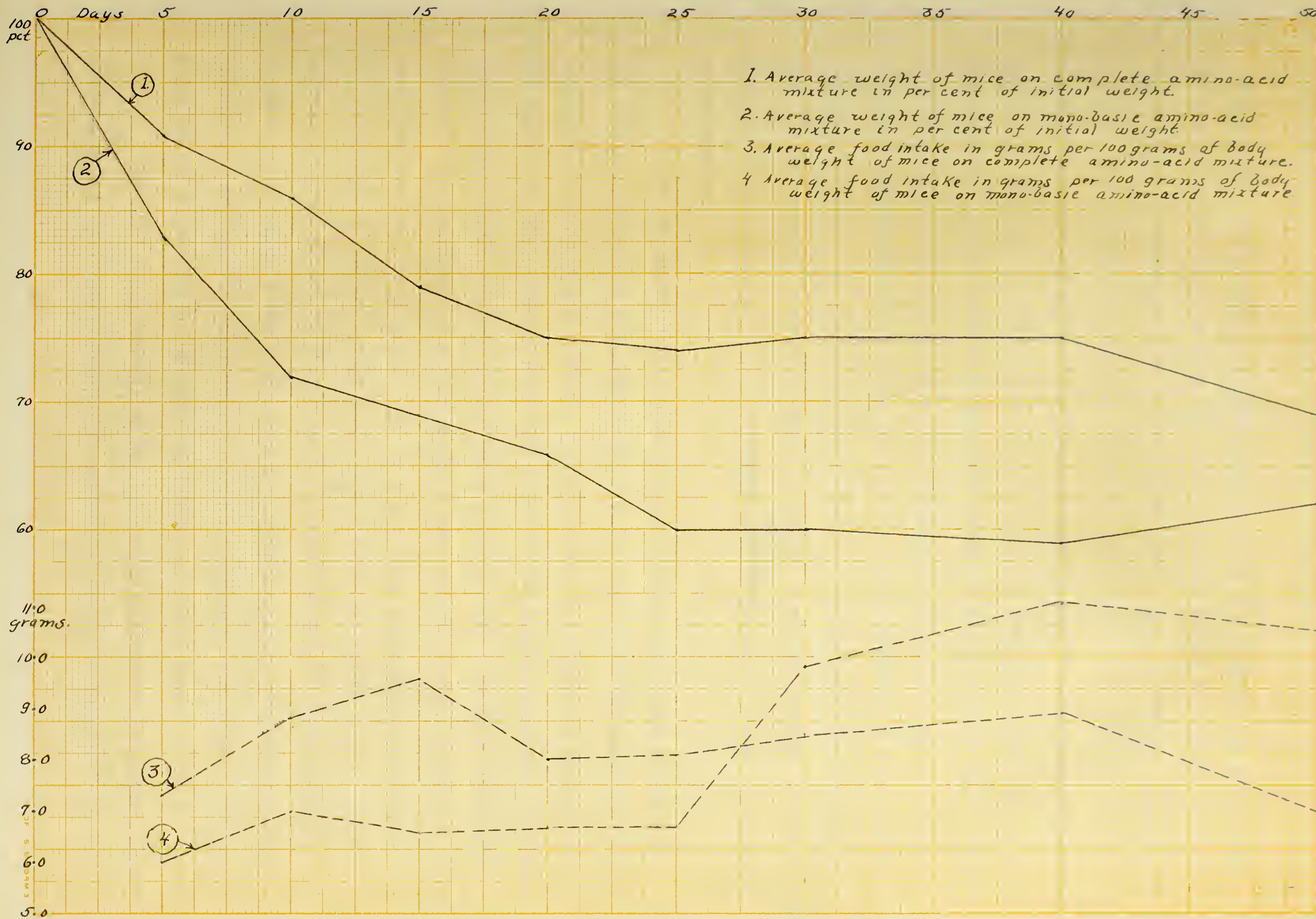
Days. Mouse.	0	5	10	15	20	25	30	40	50	75
100a ♂, 100b ♂, 100c ♂	0.0	5.5	6.3	7.6	7.5	5.8	5.8	7.3	6.5	8.5
110a ♀, 110b ♀	0.0	(1.4)'	8.0	9.3	6.1	6.0	5.9	7.4	5.1	[illegible]
140a ♂, 140b ♂, 140c ♂	0.0	8.1	10.0	10.0	7.3	10.5	11.0	11.0	9.5	10.5
101 ♂	0.0	7.4	11.6	10.8	7.9	10.2	9.4	removed.		
105a ♂, 105b ♂	0.0	---	8.1	10.5	10.3	----	10.5	10.0	died.	
Average	0.0	7.3	8.8	9.6	8.0	8.1	8.5	8.9	7.0	9.1 g.

Mice on Mono-basic Amino-acid Mixtures.

Days. Mouse.	0	5	10	15	20	25	30	40	50	75
122a ♀, 122b ♀, 122c ♀	0.0	4.9	4.3	4.7	5.6	4.2	8.6	12.8	11.7	7.0
120a ♀, 120b ♀	0.0	7.5	7.1	5.8	7.3	5.4	7.3	6.1	6.9	died.
125a ♂, 125b ♂	0.0	4.0	5.2	7.7	7.4	---	11.5	10.5	11.1	removed.
124a ♂	0.0	4.8	5.8	10.9	10.6	---	12.7	10.9	11.0	12.7
123 ♂	0.0	6.2	7.6	7.2	6.8	died.				
124 ♀	0.0	5.5	12.3	died.						
127a ♂, 127b ♂	0.0	6.5	7.6	---	7.2	7.3	6.8	9.7	died.	
128a ♂, 128b ♂, 128c ♂	0.0	5.7	8.0	---	9.3	9.8	---	5.0	12.3	died.
130a ♂, 130b ♂, 130c ♂	0.0	3.9	5.8	---	removed.					
123a ♂	0.0	10.8	6.4	9.2	9.5	---	12.0	11.8	9.9	removed.
Average	0.0	6.0	7.0	6.6	6.7	6.7	9.8	11.1	10.5	9.8 g.

'Period when mice were on calcium salts of carbamino acids; not used in the calculation.

The average food intake of the mice on the ration deficient in the di-basic amino-acids did not equal that of the mice on the ration containing the complete mixture of amino-acids until the 2[illegible]th day of the experiment. It



Days
0
5
10
15
20
25
30
35
40
45
50
100 pct.
90
80
70
60
11.0 grams.
10.0
9.0
8.0
7.0
6.0
5.0
1.
2.
3
4
1. Average weight of mice on complete amino-acid mixture in per cent of initial weight.
2. Average weight of mice on mono-basic amino-acid mixture in per cent of initial weight.
3. Average food intake in grams per 100 grams of body weight of mice on complete amino-acid mixture.
4 Average food intake in grams per 100 grams of body weight of mice on mono-basic amino-acid mixture

does not seem reasonable to assume that the mice ate less of the mono-basic amino-acid ration than the complete ration because of any difference in the taste caused by the removal of the di-basic amino-acids. However, should the di-basic amino-acids be essential, might we not expect some disorganization of the regular functions of the body in an attempt to meet the new conditions which might lead to digestive disturbances resulting in a temporary lessening of the food intake? Since the mice on the mono-basic amino-acid ration had a larger relative food intake than those on the ration containing the complete amino-acids after the 28th day, it does not seem that the inability of the mice to maintain themselves on the mono-basic ration can be ascribed to a deficient food intake, especially as an adequate (i.e. the complete) mixture of amino-acids has been shown to be capable of increasing the weight of the animal as well as maintaining it.

The average survival period for the mice on the ration containing the complete mixture of amino-acids and for the mice initially fed this ration for [illegible] to 117 days before removing to other rations, as shown by Table XIV, is 133 days. If the latter mice had been kept on the complete amino-acid ration continuously, their survival period would probably have been much longer than it was. The average time all of the mice were on the complete ration, as shown by Tables I to V, is 91 days. On the other hand, the mice kept continuously on the mono-basic ration showed an average survival of only 48 days. The average survival period of Mice 125b ♂, 130a ♂, 130c ♂, and 123a ♂, which were placed on a ration containing a complete amino-acid mixture after having been on the mono-basic ration from 11 to 72 days, was 94 days, indicating that the former ration was more efficient than the latter.

From the above results it may be concluded that the mono-basic amino-acid mixture lacked some substance or substances which are essential to the

body for long continued maintenance, and which are present in the complete amino-acid mixture used. There is no obvious explanation of the inability of the complete amino-acid mixture to maintain the mice throughout their normal period of life.

Hopkins and Foreman believe that both glutamic and aspartic acids are not indispensable to the animal organism, since they secured growth for a period of 35 days with two rats fed on a ration from which the di-basic amino-acids had been removed by the method of Foreman. There is, however, the possibility in these experiments that all of the glutamic and aspartic acids were not removed due to an incomplete hydrolysis of the protein, since, according to the determination of the alpha-amino nitrogen by the method of Van Slyke complete hydrolysis was not secured in the work reported in this paper until the protein has been boiled with ten times its weight of 25 percent sulfuric acid for a period of 50 to 55 hours. Hopkins and Foreman hydrolyzed their protein for a shorter period than this, though their statements are conflicting on this point.

In view of recent nutritional studies, it is evident that a period of 35 days is too short a time upon which to base a positive conclusion. Wheeler[a] has shown that mice, fed a diet qualitatively deficient in protein, can maintain themselves or even gain weight for 17 to 21 days before showing the ultimate decline. This period corresponds to a longer period in the life of the rat. It is also known that rats can grow for several months on a diet which lacks the fat-soluble accessory substances before the inevitable decline takes place. Had the tests of Hopkins and Foreman been carried out over a longer period of time or with larger numbers of animals, different results might have been secured.

It is to be regretted that the addition of the glutamic and aspartic acids to the mixture of mono-basic amino-acids was not begun before the animals had reached such a low state of nutrition, that their recuperation would have

difficult if not impossible with even the most favorable dietary.

The results of the investigation may be summarized briefly as follows:-

I. It was found possible to maintain mice in a normal condition for 80 to 100 days and to keep them alive as long as 208 days by feeding them a ration containing 15 percent of a mixture of amino-acids secured from the complete hydrolysis of casein or of the combined milk proteins, supplemented with tryptophane and cystine, 12 percent of sucrose, 17 percent of starch, 28 percent of "protein-free" milk, 18 percent of butter fat, and 10 percent of lard.

II. The substitution of a mixture of amino-acids from which the glutamic and aspartic acids had been removed, for the above complete mixture, resulted in a more rapid decline in weight, a shorter period of maintenance and a shortening of the survival period.

III. These differences in the survival period cannot be entirely accounted for by differences in food intake.

IV. The changing from the complete amino-acid mixture to the mono-basic amino-acid mixture always resulted in a loss in weight even tho food intake was the same. The change from the mono-basic to the complete amino-acid mixture either had the opposite effect or else checked the decline at a point where it did not ordinarily stop.

V. The material removed from the complete mixture of amino-acids as the calcium salts insoluble in alcohol is responsible for this deficiency and is necessary for the maintenance of the animal organism as indicated from these results secured with mice. It has been shown by Foreman that this material consists almost entirely of the calcium salts of glutamic and aspartic salts.

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